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## **Precision medicine in Type 2 diabetes: Clinical markers of insulin resistance are associated with altered short and long-term glycemic response to DPP4-inhibitor therapy**

### **Running title: Insulin resistance lowers response to DPP4-i's**

John M. Dennis<sup>1</sup> (MSc), Beverley M. Shields<sup>2</sup> (PhD), Anita V. Hill<sup>2</sup>, Bridget A. Knight<sup>2</sup> (PhD), Timothy J. McDonald<sup>2,3</sup> (PhD), Lauren R. Rodgers<sup>1</sup> (PhD), Michael N. Weedon<sup>4</sup> (PhD), William E. Henley<sup>1</sup> (PhD), Naveed A. Sattar<sup>5</sup> (FMedSci), Rory R. Holman<sup>6</sup> (FRCP), Ewan R. Pearson<sup>7</sup> (PhD), Andrew T. Hattersley<sup>2</sup> (DM), Angus G. Jones<sup>2</sup> (PhD) on behalf of the MASTERMIND consortium

1. Health Statistics Group, University of Exeter Medical School, Exeter U.K
2. National Institute for Health Research Exeter Clinical Research Facility, University of Exeter Medical School, Exeter, U.K.
3. Blood Sciences, Royal Devon and Exeter Hospital, Exeter, UK
4. Institute of Biomedical & Clinical Science, University of Exeter Medical School, Exeter U.K
5. Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK
6. Diabetes Trials Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK
7. Division of Molecular & Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee U.K.

### **Corresponding author**

Angus G. Jones. National Institute for Health Research Exeter Clinical Research Facility, University of Exeter Medical School and Royal Devon and Exeter National Health Service Foundation Trust, Exeter, U.K.

Address: University of Exeter Medical School. Address: Institute of Biomedical & Clinical Science, RILD Building, Royal Devon & Exeter Hospital, Barrack Road, Exeter EX2 5DW, UK

Phone: +44 1392 408538

Email: [angus.jones@exeter.ac.uk](mailto:angus.jones@exeter.ac.uk)

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## Objective

A 'precision' approach to type 2 diabetes therapy would aim to target treatment according to patient characteristics. We examined if measures of insulin resistance and secretion were associated with glycemic response to DPP4-inhibitor therapy.

## Research Design and Methods

We evaluated whether markers of insulin resistance and insulin secretion were associated with 6 month glycemic response in a prospective study of non-insulin treated participants starting DPP4-inhibitor therapy (PRIBA, n=254), with replication for routinely available markers in UK electronic healthcare records (CPRD, n=23,001). In CPRD we evaluated associations between baseline markers and 3 year durability of response. To test the specificity of findings we repeated analyses for GLP-1 receptor agonists (PRIBA n=339, CPRD n=4,464).

## Results

In PRIBA markers of higher insulin resistance (higher fasting C-peptide ( $p=0.03$ ), HOMA2 insulin resistance ( $p=0.01$ ) and triglycerides ( $p<0.01$ )) were associated with reduced 6 month HbA<sub>1c</sub> response to DPP4 inhibitors. In CPRD higher triglycerides and BMI were associated with reduced HbA<sub>1c</sub> response (both  $p<0.01$ ). A subgroup defined by obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ) and high triglycerides ( $\geq 2.3 \text{ mmol/L}$ ) had reduced 6 month response in both datasets (PRIBA HbA<sub>1c</sub> reduction 5.3[95%CI 1.8,8.6]mmol/mol (0.5%) (obese, high triglycerides) vs 11.3[8.4,14.1] mmol/mol (1.0%) (non-obese, normal triglycerides),  $p=0.01$ . In CPRD the obese, high triglycerides subgroup also had less durable response (hazard ratio 1.28[1.16,1.41],  $p<0.001$ ). There was no association between markers of insulin resistance and response to GLP-1 receptor agonists.

## **Conclusions**

Markers of higher insulin resistance are consistently associated with reduced glycemic response to DPP4-inhibitors. This finding provides a starting point for the application of a precision diabetes approach to DPP4-inhibitor therapy.

PRIBA ClinicalTrials.gov identifier: NCT01503112

Type 2 diabetes is a heterogeneous condition characterised by varying degrees of reduced beta cell function and higher levels of insulin resistance. Most of the 400 million patients worldwide will at some point require glucose lowering medication (1). Major international treatment guidelines recommend at least 4 oral treatment options after initial metformin has failed to achieve control, with choice between these informed predominantly by method of administration, overall side effect profile and cost (2-5).

Individual response to glucose lowering therapies in type 2 diabetes varies greatly. Identification of clinical phenotypic features or biomarkers robustly associated with glycemic response or other potentially beneficial effects for example reduced weight gain, or side effects for each therapy, may allow treatment of patients with the agent that is most likely to be effective for them, an approach known as 'precision' or 'stratified' medicine (6; 7). While much research has focused on identifying genetic or novel biomarker predictors of response, precision diabetes is most likely to be cost effective and have clinical impact using simple inexpensive biomarkers or routinely available clinical phenotypic features (8; 9).

DPP4 inhibitors are common (20% of U.S. and 40% of UK second-line prescriptions in 2016) (10; 11), well-tolerated (12), oral therapy options recommended in all clinical guidelines (2-5). Beyond baseline HbA<sub>1c</sub> and fasting glucose it is unclear if other factors are associated with glycemic response to DPP4 inhibitors (13; 14). A major mechanism of action of DPP4 inhibitors is potentiation of beta cell insulin secretion. We aimed to establish if measures of insulin secretion and insulin resistance were associated with short-term glycemic response and long-term durability of response in patients with type 2 diabetes starting DPP4 inhibitor therapy.

## Research Design and Methods

We assessed whether clinical features and biomarkers associated with insulin secretion and insulin resistance were predictive of short-term 6 month glycemic response in analysis of a prospective study of patients starting DPP4 inhibitor therapy as part of routine care (PRIBA). To validate our findings we tested the consistency of associations between routinely recorded factors associated with response in PRIBA in a retrospective analysis of a much larger group of patients from UK Clinical Practice Research Datalink (CPRD), evaluating both 6 month glycemic response and long term durability of response to 3 years.

### ***Study setting and assessment***

#### *PRIBA prospective study*

The PRIBA study was designed to test the hypothesis that those who have low insulin secretion, as measured by C-peptide, will have poor glycemic response to incretin based treatments (<https://clinicaltrials.gov/ct2/show/NCT01503112>), with associations between glycemic response and other clinical features, islet autoantibodies and Homeostasis Model Assessment (HOMA) 2 estimates of beta cell function and insulin sensitivity evaluated in pre-specified secondary analysis. 305 participants due to start DPP4 inhibitor therapy as part of their usual care were recruited from primary and secondary care across 17 National Institute of Health Research (NIHR) clinical research network centres in the UK from April 2011 to October 2013 as previously described (15).

At baseline (immediately prior to starting therapy) we measured HbA<sub>1c</sub>, fasting glucose, clinical markers of insulin resistance and insulin secretion (fasting C-peptide and post meal urine C-peptide Creatinine ratio (UCPCR) (16; 17); BMI, triglycerides

and HDL-cholesterol (HDL-c) (18); sex-hormone binding globulin (SHBG), GAD and IA2 islet autoantibodies) and other clinical characteristics (age at therapy, sex, duration of diabetes, eGFR, ethnicity, LDL-cholesterol (LDL-c), number of diabetes therapies). We calculated HOMA-estimates of beta-cell function (HOMA2%B) and insulin resistance (HOMA2 IR) from fasting glucose and C-peptide measures using the HOMA2 calculator available from <http://www.dtu.ox.ac.uk/homacalculator/> (19). Laboratory analysis was conducted as previously reported (15). Participants were included in the analysis if they were not insulin treated and had at least 3 months follow up with >75% adherence to therapy and limited co-treatment change (see study profile supplementary figure 1a). Ethics approval was granted by the South West National Research Ethics committee, and all participants gave written informed consent.

#### *Retrospective analysis of UK primary care patients (CPRD database)*

CPRD is the world's largest longitudinal database of anonymised primary care electronic health records (20). We included 23,001 non-insulin treated patients with type 2 diabetes with prescription records of starting a DPP4 inhibitor for the first time from June 2007 to September 2016, and followed them up whilst they remained on DPP4 inhibitor therapy without the addition or cessation of any other anti-hyperglycemic medication (see study profile supplementary figure 1b). We extracted baseline routine clinical characteristics (age at therapy, duration of diabetes, sex and BMI) and biomarkers (HbA<sub>1c</sub>, triglycerides, HDL-c, LDL-c and eGFR), with baseline defined as the most recent record in the 3 months prior to the drug start date. Ethics approval was granted by the CPRD Independent Scientific Advisory Committee (ISAC 13\_177R).

## **Analysis**

### **Outcome definitions**

#### *Short-term glycaemic response (PRIBA & CPRD)*

The primary outcome was the absolute change from baseline in HbA<sub>1c</sub> 6 months after starting therapy, adjusting for baseline HbA<sub>1c</sub>. Where a 6 month HbA<sub>1c</sub> was not available or eligible in the PRIBA study (see supplementary figure 1a) we used a 3 month HbA<sub>1c</sub> measure, as previously described (15). In CPRD a valid 6 month HbA<sub>1c</sub> was defined as the closest HbA<sub>1c</sub> to 6 months after the drug start date +/-3 months for patients on unchanged anti-hyperglycemic therapy.

#### *Durability of glycaemic response (CPRD)*

In CPRD where long-term follow-up data were available we assessed durability of response as the time to glycaemic failure over 3 years in a complete case analysis of patients with baseline HbA<sub>1c</sub> between 53-97 mmol/mol (7-11%) and at least 3 months on DPP4 inhibitor therapy (n=15,616). Glycaemic failure was defined as **a)** two consecutive HbA<sub>1c</sub>'s greater than 69 mmol/mol (8.5%) **b)** a single HbA<sub>1c</sub> greater than 69 mmol/mol (8.5%) followed by the addition of another anti-hyperglycemic therapy. To examine the sensitivity of results to this definition we repeated the analysis using HbA<sub>1c</sub> thresholds of a) 53mmol/mol (7.5%) and b) the baseline HbA<sub>1c</sub> level specific to each individual patient.

### **Statistical analysis**

#### *Short-term response (PRIBA & CPRD)*

We examined associations between each standardised marker of insulin resistance and insulin secretion and 6 month HbA<sub>1c</sub> response in a series of linear regression



models adjusted for baseline HbA<sub>1c</sub> and, in PRIBA, co-therapy change (13; 20). Non-normally distributed variables were log-transformed. We conducted a complete case analysis for each marker, including all patients with valid data even if they had missing data for other markers. To evaluate model fit we examined normality of residuals and linearity of associations for continuous variables. In both datasets we tested the independence of initial associations for each marker of insulin resistance and insulin secretion with 6 month response in further multivariable analysis, controlling for baseline HbA<sub>1c</sub> and other routinely recorded characteristics: age at therapy, duration of diabetes, sex, eGFR, LDL-c, ethnicity (CPRD only: white, non-white, missing) and co-therapy change (PRIBA only, CPRD patients all on unchanged therapy).

To further assess the robustness of findings we repeated the baseline adjusted analysis of 6 month response for males and females separately in both datasets, and in PRIBA with additional adjustment for fasting glucose. In CPRD we repeated the baseline adjusted analysis using 12 month response as the outcome in a distinct cohort of patients with 12 month (closest +/-3 months as for definition of 6 month response) HbA<sub>1c</sub> record (n=16,166).

#### *Subgroup analysis of short-term response (PRIBA & CPRD)*

Based on the initial results we defined 3 patient subgroups by standard clinical cut-offs for obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) and high triglycerides ( $\geq 2.3$  mmol/L) (21) - *Group A*: non-obese and normal triglycerides, *Group B*: non-obese OR normal triglycerides, *Group C*: obese and high triglycerides. We estimated the mean 6 month HbA<sub>1c</sub> response for each subgroup using linear regression models adjusted for baseline

HbA<sub>1c</sub> and, in PRIBA, co-therapy change. We standardised baseline HbA<sub>1c</sub> to the mean PRIBA baseline level of 74mmol/mol (8.9%) for all subgroups in both datasets.

#### *Durability of response (CPRD)*

For 3 subgroups defined by the same BMI and triglyceride thresholds we compared mean durability in response to three years after starting therapy using a flexible parametric time to failure survival model. We included all patients with at least 3 months on therapy after starting a DPP4 inhibitor with valid baseline records of all covariates (baseline HbA<sub>1c</sub>, age at therapy, duration of diabetes, sex and eGFR). The use of flexible parametric models allowed prediction of the probability of therapy failure over three years as well as hazard ratios consistent with Cox proportional hazards regression (22). We tested continuous variables for non-linearity, and evaluated proportional hazards assumptions using Schoenfeld residuals. To estimate the probability of therapy failure (the inverse of survival) for each subgroup a predicted survival curve was calculated for each patient in the dataset before the individual survival curves for all patients within a subgroup were averaged (23). Each curve was standardised to the mean CPRD values of other clinical covariates (baseline HbA<sub>1c</sub> = 72mmol/mol (8.7%), age at therapy = 64 years, duration of diabetes = 8 years, eGFR = 82 ml/min/1.73m<sup>2</sup>). Point estimates for the failure probability at 3 years by subgroup were calculated using the same approach.

#### *Replication analysis with GLP-1 receptor agonists (PRIBA and CPRD)*

To test the specificity of findings for DPP4 inhibitors we repeated the analyses of short-term response and durability of response for non-insulin treated subjects starting GLP-1 receptor agonists, the other glucose-lowering drug evaluated in PRIBA (PRIBA n=339, CPRD n=4,464). We have previously reported the PRIBA

primary analysis of predictors of glycemic response for the full PRIBA GLP-1 receptor agonist cohort, which included an additional 209 insulin treated participants (14). All data extraction and analysis were conducted using Stata v14.0 (StataCorp, College Station, TX).

## Results

### ***Patient characteristics & response to DPP4 inhibitor therapy***

Baseline characteristics and biomarker measures were similar for subjects starting DPP4 inhibitors in both datasets (Table 1). In both cohorts the majority of patients started Sitagliptin. 254 patients were included in PRIBA and 23,001 (for analysis of 6 month glycemic response) in CPRD (for study profiles see supplementary figure 1). Mean (standard deviation (SD)) 6 month HbA<sub>1c</sub> change was -8.3 (13.5) mmol/mol (-0.7% (1.2%)) in PRIBA and -7.6 (15.1) mmol/mol (-0.7% (1.4%)) in CPRD.

### ***Higher baseline fasting C-peptide and HOMA measured insulin resistance are associated with reduced glycemic response to DPP4 inhibitors***

In the PRIBA cohort mean HbA<sub>1c</sub> response was reduced by 1.67 mmol/mol for every 1 SD higher baseline fasting C-peptide (standardised  $\beta$  1.67 [95% CI 0.17 to 3.17] mmol/mol/SD,  $p=0.03$ ) (Figure 1). We observed the same direction and similar size of effect for UCPCR (response reduction per SD higher 1.65 [95% CI -0.07 to 3.37] mmol/mol,  $p=0.06$ ). Higher baseline HOMA measured insulin resistance (HOMA2-IR) was also associated with reduced response (response reduction per SD higher: 2.17 [95% CI 0.62 to 3.72], mmol/mol,  $p=0.01$ ), but there was no evidence of an association between beta-cell function (HOMA2-%B) and response (response reduction per SD higher 0.16 [95% CI -1.49 to 1.81] mmol/mol,  $p=0.85$ ). Islet autoantibody prevalence was low (2.8% GAD or IA2 positive; response reduction for presence of autoantibodies: 5.6 [95% CI -3.6, 14.7] mmol/mol,  $p=0.23$ ).

***Other markers of insulin resistance are consistently associated with glycemic response to DPP4 inhibitors in PRIBA and CPRD***

In PRIBA higher triglycerides was associated with reduced glycemic response (response reduction per SD increase 2.54 [95% CI 0.99 to 4.08] mmol/mol,  $p < 0.001$ ), with a consistent direction of association for higher BMI (response reduction per higher BMI 0.96 [95% CI -0.54 to 2.46] mmol/mol,  $p = 0.21$ ) and lower SHBG (response reduction per SD higher SHBG -1.19 [95% CI -2.81 to 0.42] mmol/mol,  $p = 0.15$ ) (Figure 1, supplementary table 1). In CPRD higher triglycerides and BMI were associated with reduced HbA<sub>1c</sub> response (Figure 1, supplementary table 1). HDL-c was not associated with response in either dataset ( $p = 0.81$  in PRIBA,  $p = 0.46$  in CPRD).

***Markers of insulin resistance are associated with glycemic response to DPP4 inhibitors independently of other routine clinical characteristics***

Results were consistent when **a)** stratifying by sex (supplementary table 1), **b)** controlling for baseline HbA<sub>1c</sub>, age at therapy, sex, duration of diabetes, eGFR, LDL-c, ethnicity (CPRD only) and co-therapy change (PRIBA only) in multivariable analysis of each dataset (supplementary table 2), **c)** in PRIBA controlling for fasting glucose (supplementary table 3) and **d)** in CPRD with 12 month HbA<sub>1c</sub> response as the outcome (supplementary table 4).

***Standard clinical criteria of obesity and high triglycerides can identify patients likely to have markedly reduced glycemic response to DPP4 inhibitors***

Higher triglycerides was associated with reduced glycemic response independently of BMI in both datasets, and higher BMI was associated with reduced response independently of triglycerides in CPRD (supplementary table 5). To examine the

potential clinical implication of this finding we compared mean baseline HbA<sub>1c</sub> adjusted response in 3 patient subgroups defined by standard clinical cut-offs for obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) and high triglycerides ( $\geq 2.3$  mmol/L) - *Subgroup A*: non-obese and normal triglycerides, *Subgroup B*: non-obese OR normal triglycerides, *Subgroup C*: obese and high triglycerides).

In PRIBA we found mean 6 month baseline HbA<sub>1c</sub> standardised glycaemic response was halved for the obese and high triglycerides subgroup (Subgroup C -5.2 [95% CI -1.8 to -8.6] mmol/mol (-0.5% [95% CI -0.2;-0.8])) compared to the non-obese and normal triglycerides subgroup (Subgroup A -11.3 [95% CI -8.4 to -14.1] mmol/mol (-1.0% [95% CI -0.8;-1.3])) and was significantly reduced compared to intermediate Subgroup B (-9.9 [95% CI -7.6 to -12.2] mmol/mol (-0.9% [95% CI -0.7;-1.1])) (Figure 2a). Direction of effect was replicated in CPRD, albeit with smaller differences in mean response between subgroups (Subgroup A mean baseline adjusted HbA<sub>1c</sub> response -10.3 [95% CI -9.8 to -10.7] mmol/mol (-0.9% [95% CI -0.9;-1.0]), Subgroup B -8.8 [95% CI -8.5 to -9.1] mmol/mol (-0.8% [95% CI -0.8;-0.8]), Subgroup C -7.5 [95% CI -7.0 to -7.9] mmol/mol (-0.7% [95% CI -0.6;-0.7]), Figure 2b).

***Obesity and high triglycerides are associated with less durable glycaemic response to DPP4 inhibitors over 3 years***

15,616 patients were followed up in this analysis for a mean time of 1.5 years. Over the 3 year study period 3,514 (23%) patients had glycaemic failure (confirmed HbA<sub>1c</sub>  $\geq 69$  mmol/mol (8.5%)). We observed an increased relative risk of glycaemic failure , reflecting a less durable response) in the same obesity and high triglycerides defined subgroups, standardising for other clinical characteristics (hazard ratios for glycaemic

failure: Subgroup C obese AND high triglycerides versus Subgroup A non-obese and normal triglycerides 1.28 [95% CI 1.16-1.41],  $p < 0.001$ ; Subgroup B obese OR high triglycerides versus Subgroup A 1.17 [95% CI 1.08-1.27],  $p < 0.001$ ; Subgroup C versus Subgroup B 1.09 [95% CI 1.01-1.18],  $p = 0.04$ ; supplementary table 6).

Consistent relative differences between subgroups were observed at HbA<sub>1c</sub> failure thresholds of 7.5% and the baseline HbA<sub>1c</sub> specific to each individual patient (supplementary table 7-8). These results translated into significant differences between subgroups in the absolute probability of glycemic failure at three years (Subgroup C: obese AND high triglycerides 39% [95% CI 37-42%]; Subgroup B: obese OR high triglycerides 37% [95% CI 35-38%]; Subgroup A: non-obese and normal triglycerides 32% [95% CI 31-34 %] supplementary figure 2).

***There is no evidence of an association between markers of insulin resistance and glycemic response to GLP-1 receptor agonists***

We found no evidence of an association between any marker of insulin resistance and 6 month glycemic response to GLP-1 receptor agonists in PRIBA ( $n = 339$ ) or CPRD ( $n = 4,464$ ) on continuous analysis (Figure 3, supplementary tables 8-9). There was also no evidence for a difference in response to GLP-1 receptor agonists across the obesity and triglyceride defined subgroups (all subgroup comparisons  $p > 0.40$ ; supplementary table 10, supplementary figure 3), although there were few subjects in the non-obese, normal triglyceride subgroup starting GLP-1 receptor agonist therapy in both datasets (PRIBA 2%, CPRD 5%). Similarly, in CPRD we found no evidence of an association between durability of glycemic response and BMI (HR per unit increase 1.01 (95% CI 1.00-1.02,  $p = 0.29$ ) or triglyceride levels (HR per unit increase 0.99 (95% CI 0.95-1.04,  $p = 0.80$ ) (supplementary table 10), or of a

difference in durability of response across obesity and triglyceride defined subgroups (supplementary table 11, supplementary figure 4).



## Conclusions

Our results show that markers of higher insulin resistance are consistently associated with reduced glycemic response to DPP4 inhibitor therapy. In our UK-representative cohort 22% of patients were obese with high triglycerides ( $\geq 2.3$  mmol/L) and these patients had both markedly reduced short-term glycemic response and shorter durability of response on DPP4 inhibitor treatment. With GLP-1 receptor agonists we found no evidence of an association between markers of insulin response and either 6 month glycemic response or durability of response to 3 years. Findings were robustly demonstrated in a prospective study and validated in real-world data and provide a starting point for the application of a precision diabetes approach with DPP4 inhibitor therapy.

Strengths of this study include that we have shown consistent findings across several clinical features and markers of insulin resistance in a prospective study and large dataset of electronic healthcare records. We have shown findings are robust with adjustment for baseline HbA<sub>1c</sub> (13; 24), and potential confounders, and by definition of glycemic response, with similar associations for short term (6 and 12 month) and long term (3 year durability) glycemic outcomes. Our study is the first to identify characteristics associated with durability of response to DPP4 inhibitor therapy, an area where evidence is limited (25).

Limitations of this study include that we were only able to partially replicate our results from the PRIBA study cohort, as measures such as C-peptide were not available in our replication dataset. Our effect size for triglycerides is notably smaller in our replication dataset. It is possible this relates to differences in triglyceride measurement (we were unable to confirm if measured triglycerides were fasted in

these real-world data) or to increased error in electronic healthcare records in comparison to the prospective study (26), or to the effect of statistical chance in the smaller dataset. The only long-term follow-up data we had to evaluate durability of glycemic response was from the routine primary care dataset CPRD, further evaluation in a trial setting with greater follow-up than PRIBA would be of considerable interest. An additional important limitation is that this study has examined response to only two of the available therapies. Evidence is limited for other therapies, although a previous study found no evidence of a relationship between clinical insulin resistance or dyslipidaemia markers and glycemic response with the SGLT-2 inhibitor Dapagliflozin (27). High BMI and triglycerides have both been shown to be associated with modest increases in the rate of diabetes progression (28). While this is unlikely to be relevant to our finding for 6 month glycemic response this could influence our findings for treatment durability, and replication looking at other comparison therapies is therefore particularly important in this context. While we have only examined relatively crude measures of insulin resistance, for clinical practice we consider it very unlikely that more complex measures would ever be feasible (29).

Existing studies of the association between insulin resistance and short-term glycemic response to DPP4 inhibitors have not shown consistent findings and are constrained by methodological and reporting limitations, as recently reviewed by Bihan and colleagues (13). Meta-regression of study level data have suggested reduced glycemic response in patients with higher BMI in one study (30), but no relationship in another analysis (31). These studies should be interpreted with some caution due to risk of ecological bias (32; 33). A number of individual clinical trials of DPP4 inhibitors have commented on consistency of glucose response across

subgroups defined by baseline BMI or insulin resistance, reduced glycemic response with high HOMA measured insulin resistance was reported in 2 of 7 studies, and reduced response with high BMI in 6 of 36 studies, as reviewed in Bihan et al (13). No studies reported an opposite direction of effect. These reports are very limited, with the vast majority providing no statistical comparison or details of what analysis was undertaken. An important issue for analysis of this nature is accounting for the influence of baseline HbA<sub>1c</sub>, the strongest predictor of glycemic response, which may confound true associations, especially as baseline HbA<sub>1c</sub> and insulin resistance are positively correlated (24; 34; 35). There are limited data examining the relationship between triglycerides and response to DPP4 inhibitors, however one study stratified patients by baseline triglycerides ( $\leq$ 1.7mmol/l) and found the odds of achieving an HbA<sub>1c</sub> target of 53mmol/mol (7%) were doubled in the low triglyceride subgroup (OR 2.2 [95% CI 1.0-4.7],  $p=0.04$ ) (36).

While it is plausible our finding of reduced glycemic response in those with high BMI or high triglycerides directly relates to insulin resistance through reduced effect of drug potentiated insulin secretion, this effect is not apparent in other drugs with effects on insulin secretion, for example there is no relationship between obesity and response to sulfonylurea therapy or GLP-1 receptor agonists (15; 37). An alternative explanation would be a direct effect of lipotoxicity, or indirect associations with other (unmeasured) factors important to DPP4 inhibitor response. A direct mechanism for lipotoxicity in reducing response to incretin based therapy has been previously suggested, with expression of GLP1 receptors diminished in islets exposed to elevated fatty acid levels in animal models, and beta cell response to GLP1 restored following fatty acid reduction with fibrate pharmacotherapy, however this mechanism would not explain the lack of an association between these features and GLP-1

Receptor Agonist response(38). It has also been shown that GLP-1 response is blunted in obese insulin resistant patients with high liver fat and also blunted in patients with high fasting triglycerides (39; 40), therefore impaired GLP-1 secretion in obese insulin resistant individuals represents a potential indirect mechanism that could also account for the lack of a similar relationship for injected GLP-1 receptor agonist therapy. While the lack of association for HDL-c may be considered unexpected, we note HDL-c has a much weaker relationship with insulin resistance than either triglycerides or fasting insulin/C-peptide, which may explain this finding (18).

Our findings have potential implications for clinical practice, as both BMI and triglycerides are routinely available at no additional cost. Stratification of treatment based on these criteria may therefore be cost effective even with the more modest differences in treatment effect seen in our replication cohort. Although our own and previous research suggests these findings may be specific to DPP4 inhibitors further work examining the relationship between these, and other, factors and response to comparator drugs is needed. Our study design, emphasising the importance of replication across datasets, provides an exemplar for such future analyses. In addition while simple categorisation by subgroup may provide a starting point for prediction of therapy response in type 2 diabetes, we anticipate a more sophisticated precision diabetes approach combining features into a multivariable response calculator will have greatest clinical utility, and this is an important area for future research (8; 41).

In conclusion, our study shows simple markers of higher insulin resistance are consistently associated with reduced glycemic response to DPP4 inhibitor therapy. This finding was robustly demonstrated in a prospective study and validated in real-

world data and provide a starting point for the application of a precision diabetes approach to DPP4 inhibitor therapy in type 2 diabetes.

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## Conflict of Interest statement

WEH declares a grant from Quintiles, ERP declares personal fees from Lilly, Novo Nordisk, and Astra Zeneca. NS declares personal fees from Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Janssen and a grant from Astra Zeneca. RHH declares research funding from Bayer, Astra Zeneca, MSD and honoraria from Amgen, Bayer, Elcelyx, Janssen, Intarcia, MSD, Novartis, Novo Nordisk and Servier. For all other authors there are no other potential conflicts of interest relevant to this article.

## Author Contributions

Contributors: AGJ and JMD designed the study. AGJ, AVH, BAK and TJM researched the data (PRIBA study), BMS, LRR and MNW extracted the CPRD data. JMD, BMS and AGJ analysed the data with assistance from WEH. JMD and AGJ drafted the article. ATH, ERP, NS, WEH, and RHH discussed and contributed to study design, provided support for the analysis and interpretation of results. All authors critically revised the article and approved the final version. All authors had full access to all of the data and take responsibility for the integrity of the data and the accuracy of the data analysis.

## Guarantor

AGJ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Table 1: Subject baseline characteristics**

<b>Characteristics</b>		<b>PRIBA (n=254)</b>	<b>CPRD (n=23,001)</b>
mean (SD) unless stated			
Baseline HbA1c (mmol/mol)		74 (12)	72 (15)
Baseline HbA1c (%)		8.9 (1.1)	8.7 (1.3)
Age at therapy start (years)		63 (10)	64 (11)
Age at diagnosis (years)		54 (10)	56 (10)
Male sex (%)		63%	61%
Duration of diabetes (years)		9 (6)	8 (5)
BMI - median (IQR); mean(SD)		32 (29-37); 33 (6)	32 (28-36); 33 (6)
Ethnicity (%)			
	White	97%	45%
	Non-White	3%	6%
	Missing	0%	49%
<b>Biomarkers</b>			
median (IQR); mean (SD) unless stated			
*=log-transformed			
Triglycerides (mmol/L)		1.7 (1.2-2.4); 1.8 (0.9)*	1.8 (1.3-2.6); 1.9 (1.0)*
HDL-c (mmol/L)		1.1 (0.9-1.3); 1.1 (0.3)*	1.1 (0.9-1.3); 1.1 (0.3)*
LDL-c (mmol/L)		1.9 (1.5-2.3); 1.9 (0.8)*	2.1 (1.6-2.6); 2.0 (0.8)*
SHBG (nmol/L)		27 (19-41); 27 (16)*	NA
Fasting C-peptide (pmol/L)		1150 (820-1460); 1090 (480)*	NA
HOMA2-%B		54 (37-73); 51 (27)*	NA
HOMA2 IR		3.1 (2.3-4.2); 3.1 (1.5)*	NA
UCPCR nmol/mmol		3.4 (2.0-5.0); 3.0 (2.3)*	NA
eGFR (ml/min/1.3m <sup>2</sup> )		85 (70-98); 85 (24)	82 (66-97); 82 (23)
GAD or IA2 positive (%)		3%	NA
<b>Therapy</b>			
Number of concomitant therapies at therapy start (% of total)			
	0	3%	6%
	1	35%	51%
	2	57%	42%
	3+	5%	2%
DPP4 type (% of total)			
	Sitagliptin	87%	72%
	Alogliptin	0%	2%
	Linagliptin	4%	10%
	Saxagliptin	6%	12%
	Vildagliptin	2%	4%

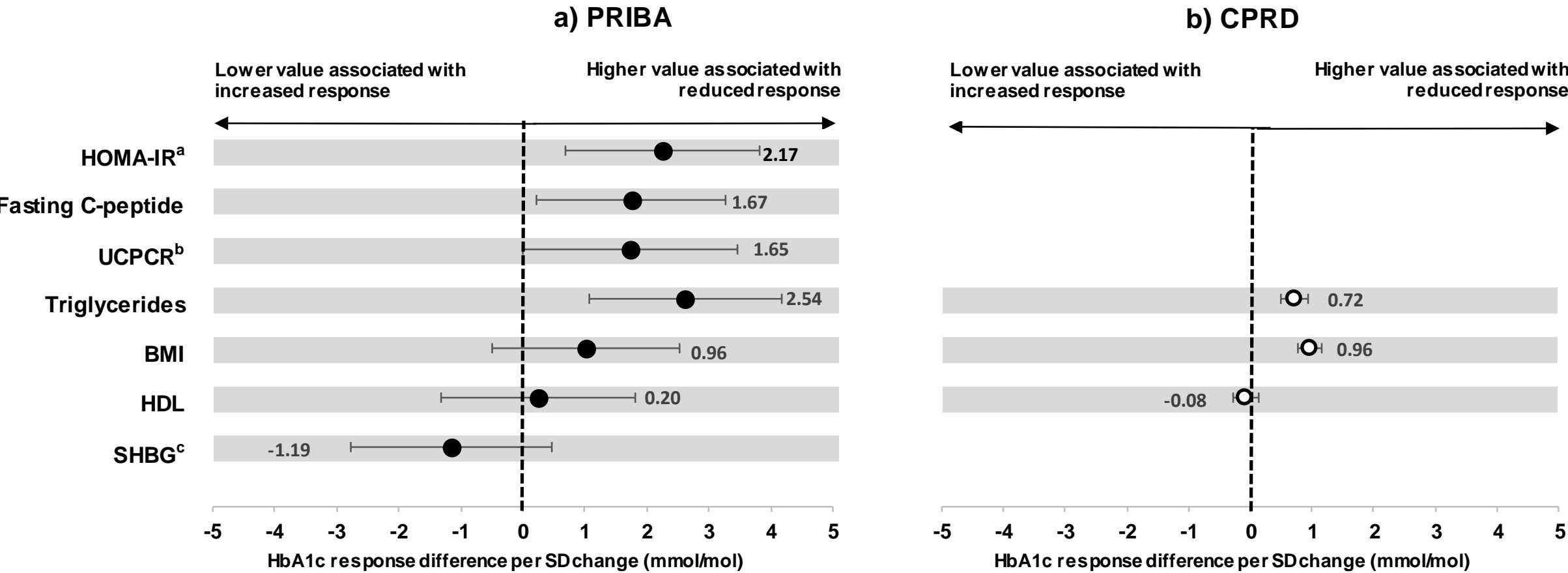
## Figure legends

**Figure 1: DPP4 inhibitors - associations between markers of insulin resistance and HbA1c response at 6 months.** Circles (black = PRIBA, white = CPRD) denote the mean HbA1c change (mmol/mol) at 6 months per 1 standard deviation (SD) higher baseline value of each marker. Error bars denote 95% confidence intervals.

**Figure 2: DPP4 inhibitors - predicted mean absolute HbA1c change from baseline at 6 months in a) PRIBA b) CPRD across subgroups defined by the presence or absence of obesity ( $BMI \geq 30 \text{ kg/m}^2$ ) and high triglycerides ( $TRGs \geq 2.3 \text{ mmol/L}$ ) - *Subgroup A: non-obese and normal triglycerides, Subgroup B: non-obese OR normal triglycerides, Subgroup C: obese and high triglycerides.* Baseline HbA1c is standardised to the mean PRIBA baseline level of 74mmol/mol (8.9%) for all subgroups. Error bars denote 95% confidence intervals.**

**Figure 3: GLP-1 receptor agonists - associations between markers of insulin resistance and HbA1c response at 6 months.** Circles (black = PRIBA, white = CPRD) denote the mean HbA1c change (mmol/mol) at 6 months per 1 standard deviation (SD) higher baseline value of each marker. Error bars denote 95% confidence intervals.

Figure 1



<sup>a</sup> HOMA2 measured insulin resistance <sup>b</sup> UCPCR = post meal urine C-peptide Creatinine ratio <sup>c</sup> SHBG = sex-hormone binding globulin

# Figure 2

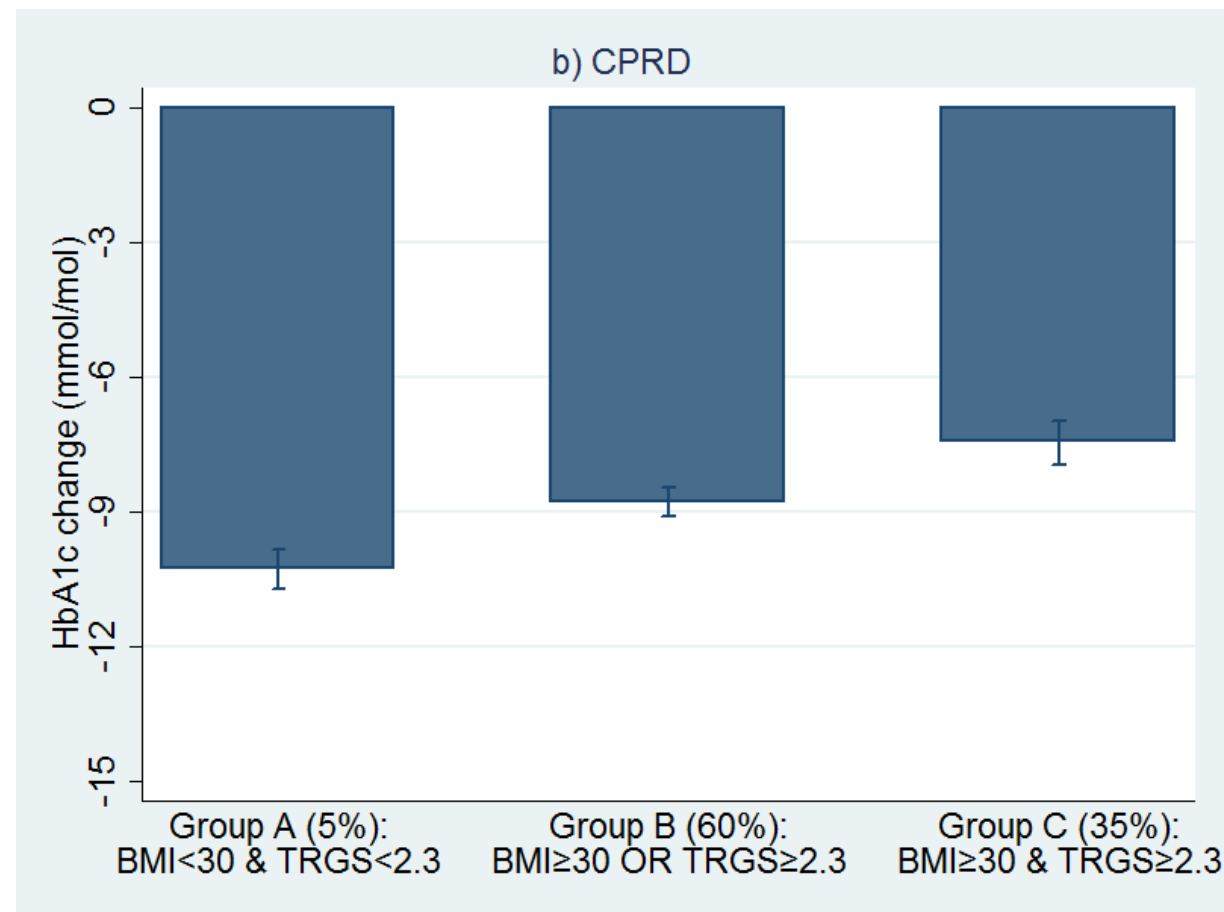
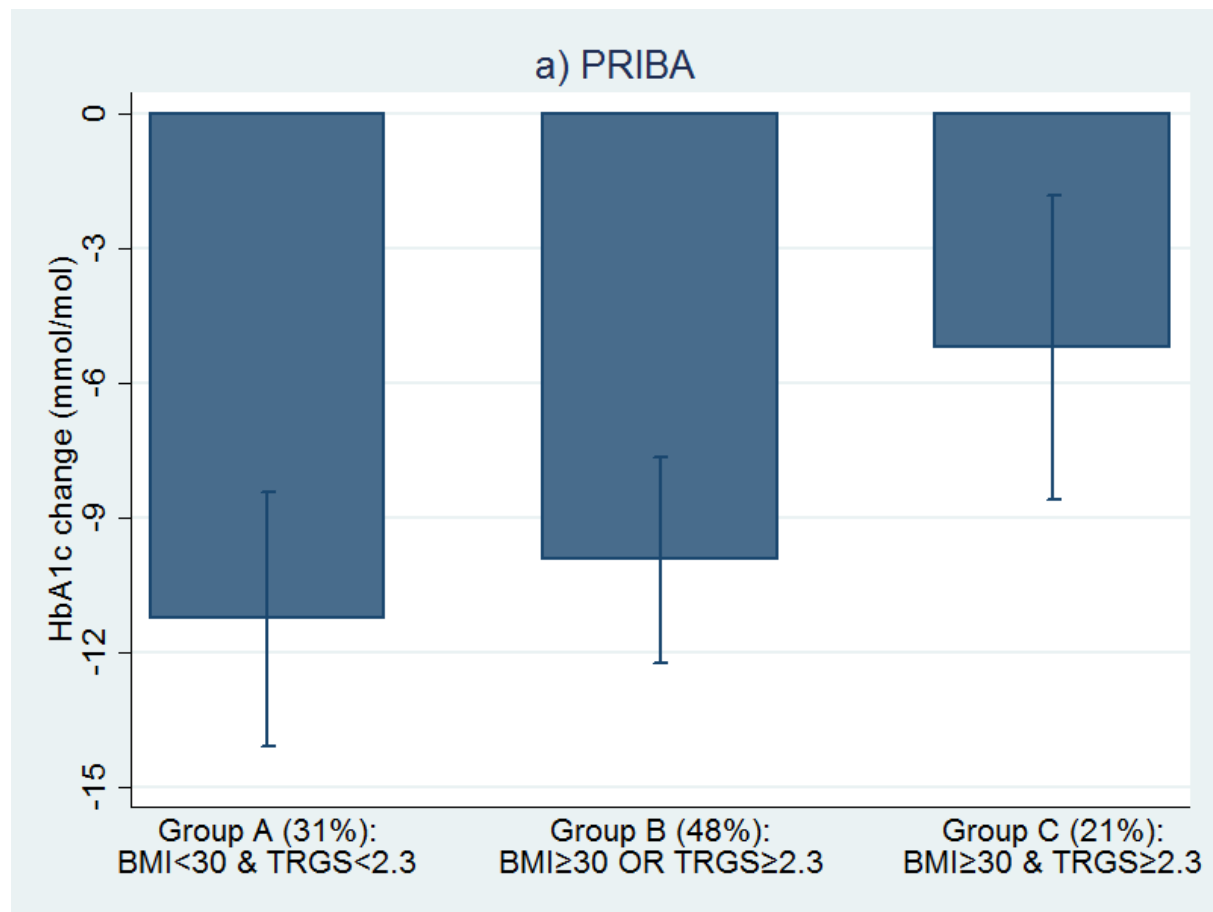
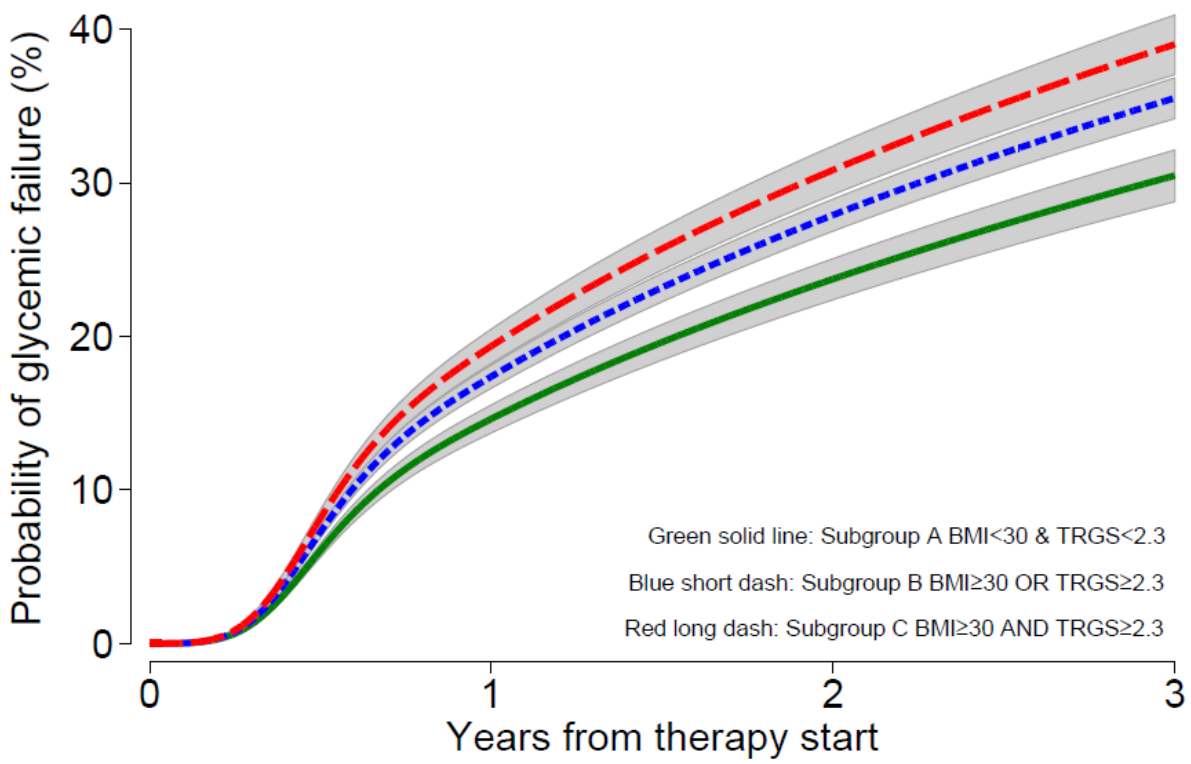
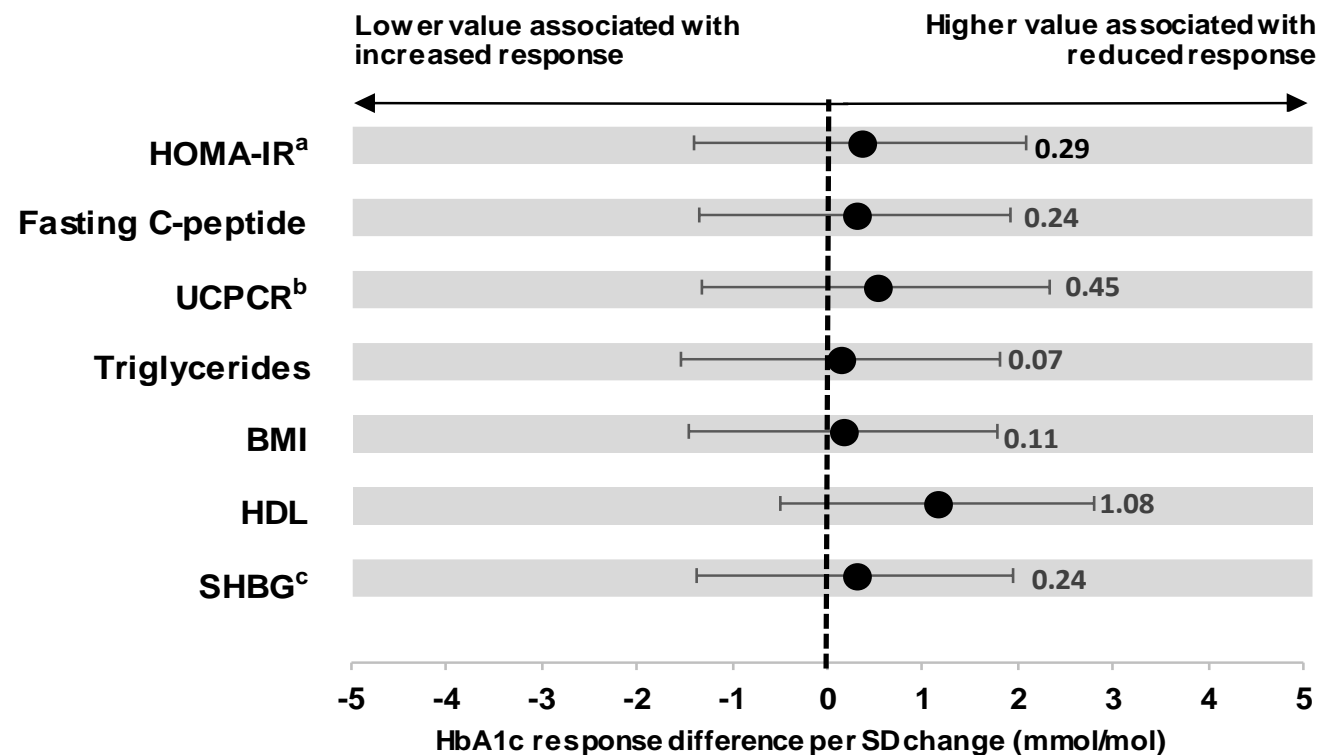


Figure 3

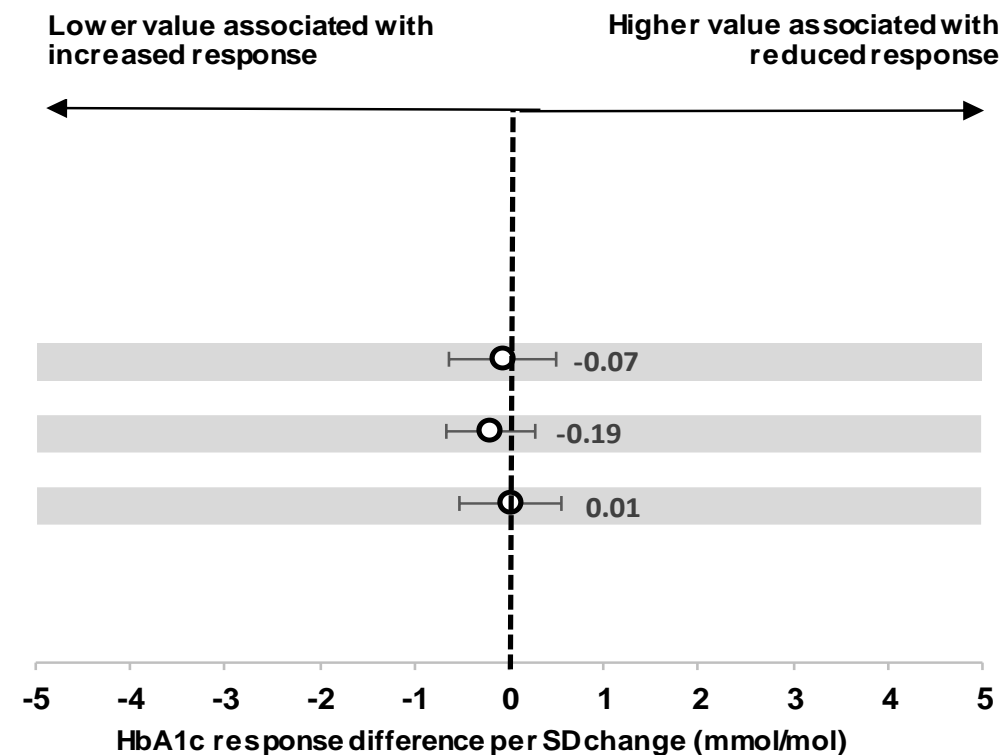


# GLP-1 R Figure

a) PRIBA



b) CPRD



<sup>a</sup> HOMA2 measured insulin resistance <sup>b</sup> UCPCR = post meal urine C-peptide Creatinine ratio <sup>c</sup> SHBG = sex-hormone binding globulin